**Case 5: X-linked Severe Combined Immunodeficiency (SCID)**

**Summary:** SCID is caused by an absence of functional T cells. Children born with SCID seem normal for first few weeks or months and then begin to acquire infections, often with opportunistic pathogens, and die without appropriate treatment while in infancy. Called severe because its fatal and called combined because in humans B ceels can’t function without help from T cells, so even if the B cells aren’t directly affected by the defect, both humoral and cell mediated immunity are lost. Phenotype that can be caused from many different genetic defects. Incidence is 3 times greater in males because the most common form is X linked (55% of cases in US). Medical emergency. Fatal unless reconstitiution of T cell immunity is achieved.

First symptoms are those of thrush in the mouth and diaper area, persistent cough from pneumocystis jirovecii infection, intersitial pneumonia due to viral infection (adenovirus, RSV, PIV, CMV), and intractable diarrhea (enterpathic coliform bacilli or viruses).

In all common forms of SCID the thymus fails to become a central lymphoid organ due to lack of invasion of precursor T cells, into the thymus gland during gestation. Patients have small dysplastic thymus. No cortico medullary differentiation, Hassall’s corpuscles are absent. Diagnosis also based on the enumeration, phenotypic characterization, and functional analysis of circulating lymphocytes, also mutation analysis.

4 categories of SCID defects:

1. Defects that impair lymphocyte survival: adenosine deaminase deficiency (adenosine metabolites that are toxic to T and B cells accunulate), reticular dysgenesis (increased apoptosis of T lymphocyte progenitors and myeloid precursors, extreme lymphopenia, absence of neutorphils, and sensoineural deafness, mutations in adenylate kinase 2 gene that regulates intracelllular levels of ADP)
2. Defects in cytokine mediated signals for lymphocyte maturation and proliferation: includes x-linked form IL2RG gene that codes the common gamma chain (CD132) that is shared by IL-2R and other cytokine receptors (IL4R, IL7R, IL9R, IL15R, IL21R), gamma chain associated with intracellular tyrosine kinase JAK3 (encoded by an autosomal gene), JAK3 is essential for intracellular signaling mediated by all gamma-containing cytokine receptors
3. Defecs in machinery for somatic gene rearrangment during lymphocyte development: RAG genes for the lymphocyte-specific recombinase and defects in ubiquitously expressed DNA repair genes also involved in recombination
4. Mutations in genes that encode CD3 molecules that particupate in formation of CD3:TCR comple to allow signaling through the pre-T cell receptor
5. Other forms that are due to defects that affect later stages in T cell development, residual presence of circulating T lymphocytes

In patients with ADA and RAG deficiencies both T and B cells are absent but NK cells aren’t affected. In patients with C linked SCID there are no T cells but B cell numbers are normal and NK cells are absent. Patients with JAK3 deficiency also lack T and NK cells and have normal number of B cells. Patients with IL7R gene defect have no T cells but normal B and NK cells. Patients with mutations in genes for CD3 chains have T cell deficiency. Patients who lack the tyrosine kinase ZAP 70 have issues transducing signals from the TCR (needed to activate TFs NFkB NFAT and AP1 to act in nucleus and initate gene transcription to result in differentiation, proliferation, and effector T cell action) and lack circulating CD8 T cells (CD4 T cells develop normally but are unable to proliferate in response to mitogens and antigens), STIM1 protein defects (senses levels of Ca stores in the ER( and ORAI1 (involved in formation of calcium released activated calcium channales) permit thymic T cell development but peripheral T cells fail to respond to activating signals (SCID symptoms as well as myopathy and autoimmune manifestations).

Common gamma chain is needed for the IL7R, which has a critical role in T cell development. Absence of NK cells in X linked SCID and JAK3 deficiency patients is due to impaired signaling through IL15R

B cells (even after transplant) are impaired in their response to IL4 (for class switching( and IL21 (produced by fillicular helper T cells to promote germinal center B cell reaction and termination differentiation of B cells)

**Case:** 3 month old boy, runny nose and persistent dry cough, middle ear infection, recurrence of otitis media, pneumonia in both lungs, thrush (candida) in mouth, red rash in diaer area, not gaining weight, normal WBC count but none of his lymphocytes reacted with anti-CD3 (no T cells), 99% bound Ab against B cell molecule CD20, 1% were NK cells reacting with anti CD16, undetectable serum IgA, blood mononuclear cells completely unresponsive to Ag, red cells has normal amounts of ADA and purine nucleoside phosphorylase, sputum culture showed RSV, mom’s blood sample showed T cells that exhibited complete nonrandom X chromosome inactivation, deletion of single NT in IL2RG gene

**Treatment:** purified CD34 cells from mother’s bone marrow (purify to prevent graft vs. host disease, CD34 is expressed on heatopoietic stem cells and progenitors, selection depletes mature T cells), IV gamma globulin, Hematopoietic cell transplantation is treatment of choice (use HLA matched family donor and cure can be achieved in more than 90% of infants, partially matched haploidentical family donors or unrelated donors can be used and allow survival in 60-80% of cases), HCT can correct Ab deficiency in some cases but if not immunoglobulin replacement therapy must reamin, gene therapy (retroviral vectors to allow expression of gamma chain) has been successful in some patients but leukemic proliferation is possible (results from activation of oncogenes at site of retroviral integration).

**Explain that SCID occurs when there is a lack of functional T cells.**

**Explain the most common form of SCID is X-linked, so SCID is seen in boys more commonly than in girls.**

**Describe the normal pattern of steps for T cell maturation, leading to the expression of functional TCRαβ on naïve T cells in the thymus, and also describe the cellular organization of the thymus.**

Cortex: immature thymocytes, branched cortical epithelia cells, scatter macrophages, outer cortical thymocytes are proliferating immature cells and the deeper thymocytes are undergoing thymic selection

Medulla: mature thymocytes, medullary epithelial cells, macrophages and dendritic cells of bone marrow origin

**Explain that neither medulla:cortex differentiation nor Hassall’s corpuscles are found in the thymus of children with SCID.**

**Explain that chronic thrush (candidiasis of the mucosa) and *Pneumocystis* pneumonia are associated with severe depression of T cell function, such as is found in SCID.**

P. jirovecii organisms are present in lung fluid and pulmonary macrophages. Don’t incite an inflammatory reponse until the infant has T cells bearing a Ag receptor for the pathogen. After successful transplant the transplanted T cells recognize the Ag and incite an inflammatory response which makes the pneumonia more severe (treat with Ab prophylatically)

**Describe the lab use of mitogens (often plant lectins) to induce division of mature lymphocytes and that, of these, phytohemagglutinin (PHA), concanavalin A (ConA) and pokeweed mitogen (PWM) are most widely used to detect T and B cell function.**

**Explain why the mothers of boys with X-linked SCID have non-random inactivation of the X chromosome in their T cells.**

In somatic cells of females 1 of the X chromones is inactivated so each is normally active in 50% of cells. If the normal X chromosome is inactivated in a BTK carrier in a pre B cell, that cell has no normal BTK gene product and can’t mature. Therefore, all of their B cells have the normal X chromosome active (nonrandom activation). Could use a marker that allows us to distinguish between the 2 X chromosomes and determine whether the B cells exhibit random or nonrandom X inactivation.

**Describe the cause of X-linked SCID as a defect in a  chain that is common to several interleukin receptors.**

IL2 (peripheral T cell homeostasis, in resting mature T cells only the beta and gamma chains of the R are present which bind IL2 with moderate affinity and allow T cells to respond to very high concentrations, activation infuces alpha chain synthesis and the formation of the heterotrimeric rec which has high affinity for IL2 and allows the T cell to respond to very low concentrations of IL2), IL4 (class switching), IL7 (T cell development), IL9 (hematopoesis), IL15 (NK cell development), IL 21 (B cell maturation)

**Explain that bone marrow transplantation is used to reconstitute immune function in SCID and describe why mature T cells in the bone marrow graft need to be removed (i.e. they can cause graft-*vs*-host disease).**

**Explain why SCID babies must NEVER be given live vaccines.**

Are unable to fight off the infection and mount an immune response, can die from the complications (ex: vaccinia given would progress to infection, vaccinia gangrenosa and was fatal)

**Case 6: Adenosine Deaminase (ADA) Deficiency**

**Summary:** Autosomal recessive. Encdoed in chromosome 20. Almost complete absence of both B and T cells when enzyme is inactive or deficient. ADA is used in purine degradation. Ubiquitous housekeeping enzyme found in all mammalian cells and blood serum. Converts purine nucleosides adenosine and deoxyadenosine to inosine and deozyinosine which are then excreted as uric acid. The limited amounts of adenosine are converted to AMP, ADP, and ATP (same with deoxy but dAMP, dADP, dATP). In its absence cells can accumulate excessive amounts of posphorylated adneosine and deoxyadenosine metabolites which are toxic when present in excessive amounts (especially to lymphocytes). Excess dATP in particulat inhibits ribonucleotide reductase which is needed for the synthesis of all deocynucleotides required for DNA synthesis (inhibition is probably main culprit in causing death and nondevlopment of lymphocytes). Leads to SCID and other extraimmune manifestations (due to its ubiquitous expression).

Thymus poorly developed. Thymus contains 13 times more ADA than any other tissue (because adenosine metabolites are so toxic to lymphocytes the high levels of ADA in the thymus are probably crucial for normal thymocyte development). Lymphocytes are particuarly vulnerable because they are relatively deficient in the enzyme 5’ nucleotidase (degrades AMP and dAMP to adenosine and deoxyadenosine and prevents the excessive accumulation of ADP, ATP, dADP, and dATP even in the absence of ADA). Lack of thymic shadow is helpful but not reliable because the thymus can shrink due to many things (stresses, infections).

Characteristic rib flaring and other bone changes. Sensorineural deafness, neurological and/or behavioral problems (may persist eve after treatment).

Purine nucleoside phosphorylase is an enzyme that degrades guanosine to inosine, absence results in excess of guanosine metabolites and deoxyguanosine metabolites which are also toxic to lymphocytes. Can also lead to SCID. T cell lymphopenia and number of circulating B lymphocytes is variable. Progressive neurological deterioration and autoimmune manifestations (autoimmune hemolytic anemia) are comme. HSCT is the only curative treatment.

**Case:** parents that are probably distantly related, previous son developed severe pneumonia at 3 months and died (had SCID), daughter seemed healthy at birth but developed thrush in her mouth at 6 weeks, no thymic study and A margin of ribs were flared on chest X ray, low lymphocyte counts and they didn’t respond to nonspecific T cell mitogen phytohemagglutinin), bone marrow donation from HLA and blood type identical brother lead to increased lymphocyte count (all responding lymphocytes were of XY origin)

**Treatment:** hematopoietic stem cell transplantation from HLA identical family donor is treatment of choice (only available for 10-15% of patients), HLA-mismatched donors have significant mortality and incomplete immune reconstitution, administration of ADA bound to polyethylene glycol clears metabolites and results in improved immune function, gene therapy (patients bone marrow CD34 hematopoietic stem cells transfuced in vitro with retroviral vector with normal ADA gene, reinfuse into patient after nonmyeloablative regimen) \*NOTE: ADA deficient patients often remain with low normal count of circulating T lymphocytes even if full detoxification is attained (accumulation of toxic metabolites can cause irreversible damage to the thymus so full restoration of T cell generation may not ever be possible)

**Explain that SCID occurs when there is a lack of functional T cells and that there are multiple conditions that can lead to the SCID phenotype.**

**Explain that deficiency in ADA leads to SCID because of buildup of the toxic intermediates, adenosine and deoxyadenosine, which are normally metabolized to uric acid but which are particularly toxic in lymphocytes. Both B and T cells are very susceptible to adenosine and deoxyadenosine.**

**Describe that SCID due to ADA deficiency can be differentiated from X-linked SCID by the absence of B cells.**

**Explain how the mixed lymphocyte reaction is performed and interpreted, and why one set of lymphocytes is either treated with mitomycin C or irradiated to ensure it acts solely as a stimulator rather than being able to proliferate.**

Used to detect histoincompatibility. The cells from the recipient (which also contain Ag presenting cells) are treated with mitomycin C (in SCID you don’t have to because they can’t respond) so they act as stimulator cells but can’t respond by DNA synthesis and cell division to Ag stimulation by the other person’s cells. Mix the cells from the 2 people. If the unirradiated lymphocytes contain alloreactive T cells these will be stimulated to proliferate and differentiated to effector cells. 3-7 days later the culture is assessed for T cell proliferation to check for CD4 T cells (using incorporation of radioactive thymidine) that recognize difference in MHC2 and for Tc cells (labeled with Cr) which respond to differences in MHC1

Prospective donor’s cells are used as the responder cells and the prospective recipients cells are the stimulator cells

**Explain why mitomycin C treatment or irradiation of a SCID recipient’s cells is not required for mixed lymphocyte reactions when testing a donor’s lymphocytes for responsiveness to SCID cells**

T cells of a SCID patient are incapable of responding to a mitogenic stimulus (patient’s cells can’t respond to potential donors cells)

**Explain why SCID babies must NEVER be given live vaccines.**

**Case 7: Omenn Syndrome: Partial enzymatic activity of RAG-1 or RAG-2**

**Summary:** RAG1 and 2 enzymes function in VDJ recombination to nick dsDNA. Recognize recombination signal sequences, which flank the coding gene segments. RAG1 binds to a nonamer and RAG2 binds and nicks the hepatmer. Coding ends are initially sealed by a hairpin and a series of ubiquitously expressed proteins (Ku70 and 80, DNA PKcs, Artemis, DNA ligase IV, XRCC4, and Cernunnos/XLF) are recruited to mediate DNA repair and rejoing of the coding and signal ends. Permits gene segment assembly to make the variabel sequence that encodes the V regions of heavy and light chains of Igs and alpha and beta chains of TCRs.

Full defects of RAG enzymes in SCID patients lead to a lack of both T and B cells. Defects in Artemis, LIG4, and DNA PK have also been identified in T and B cell lacking patients and mutations in Cernunnos/XLF lead to reduced T and B cells. In addition to T-B-NK+ SCID patients with defects in Artemis, DNA PK, LIG4 and Cernunnos genes patients present increased cellular sensitivity to ionizing radiation because they are unable to repair radiation induced DNA damage (often associated with extraimmune clinical manifestations such as microcephaly, neurodevelopmental problems, and growth and development defects).

Hypomorphic mutations can allow residual protein expression and function, resulting in a different phenotype in which autoimmune manifestations associate with severe immunodeficiency: Omenn syndrome, most often due to missense mutations in RAG genes. Partial enzyme activity expressed.

Characterized by early onset of generalized red rash (erythroderma), failure to rhive, protracted diarrhea, enlargement of liver, spleen, and lymph nodes, high eosinophil count, lack of B cells and decreased T cells. Immunogloblinas are decreased but IgE levels are raised. Only partial ability to execute VDJ recombination is retained by the mutated enzyme so in most cases no mature circulating B cells are detected and the few T cells that are found are oligoclonal (products of a limited number of different clones). Oligoclonal T cells infiltrate and cause significant damage in target organs. This is due to poor generation of T cells in the thymus resulting in impaired maturation of medullary thymic epithelial cells and reduced AIRE expression which impinges on the deletion of self reactive T cells. Also, generation of reg T cells in the thymus is also impaired which impacts peripheral tolerance. Also the few T cells that are generated in the thymus undergo extensive peripheral expansion (homeostatic proliferation) and secrete increased amounts of cytokines (IFNg, IL4, IL5).

Other causes of Omenn syndrome: IL7Ralpha chain deficiency (IL7 required for lymphocyte development), gamma chain deficiency (Xlinked SCID), and mutations of the RMRP gene (causes cartilage hair hypoplasia characterized by dwarfism, sparse hair, immunodeficiency and hematological abnormalities). Occur when the defect is leaky (missense mutations that severely impair but don’t abolish function and allow few T cells to develop).

**Case:** had brother and sister that were affected but parents weren’t (autosomal recessive inheritance) at birth seemed normal, soon after 10 loose BMs a day, rash on legs that spread over entire body, dry cough, diffuse papular scaly rash was worst on face but also covered trunk and extremitis, small blisters present on palms and soles which were red, purulent conjunctivitis (yellow discharge from eyes), initially no enlarged lymph nodes, normal heart and lungs, liver and spleen not enlarged, Hb was low, platelets slightly elevated, normal WBCs, elevated eosinophils and monocytes, low neutrophils and lymphocytes, bone marrow showed preponderance of eosinophil precurors, low IgE, higher IgE, undetectable IgA and IgM, skin biopsy showed infiltration of dermis with eosinophils, lymphocytes, and macrophages, cells surrounded BVs, no thymic shadow on chest X ray, condition worsened and enlarged lymph nodes, staph and candida cultured from drainage behind ear, thrush in mouth, no B cells and few T cells, peripheral blood lymphocytes responded poorly to stimulation with phytohemagglutinin and with anti CD3 Ab, no cells reacted with anti CD19 (detects B cells), all lymphocytes were CD3 with activation markers CD45R0 and MHC II molecules, 80% were CD4 and 15% CD8, FCAS showed only few of them were expressed (olgioclonal), RAG genes showed mutations, died from pneumocystis (respiratory failure)

Enlarged lymph nodes were due to the activation of the few clones of T cells that were able to mature (shown by surface expression of CD45R0 and MHC class 2 molecules), activated clones expanded within the lymph nodes.

**Treatment:** usually fatal unless treated by bone marrow transplantation (may result in full correction)

**Describe the essential roles of RAG-1 and RAG-2 (recombination activation gene products) in enabling rearrangement of germline DNA to form a VDJ or VJ component of the gene encoding the antigen receptor (Ig in B cells and TCR in T cells).**

**Explain that SCID occurs when either RAG-1 or RAG-2 is completely inactive, which results in the lack of T and B cell development; i.e. no functional T and B cells.**

**Explain that in Omenn syndrome there are mutant forms of RAG-1 or RAG-2, resulting from missense mutations in the *RAG-1*and *RAG-2* genes. The mutant enzymes show partial activity and this can lead to the formation of a very restricted repertoire of antigen specificities. These few antigen receptors are inadequate to give protection from pathogenic microbes, so babies develop a severe combined immunodeficiency (SCID).**

**Describe the necessity for bone marrow transplants to rescue such babies.**

**Explain why eosinophilia and elevated serum IgE are usually seen in Omenn syndrome.**

The few T cells that are produced and activated must have Th2 phenotype and secrete large amounts if IL4 (class switching to IgE) and IL5 (recruitement of eosinophils). The few B cells that the patient have (below the limit of detection) must have been induced to switch immunoglobulin class to IgE.

**Describe the role of IL-4 to enable B cells to switch to IgE synthesis, and for IL-5 to allow recruitment/development of eosinophils. Describe the cellular source for IL-4 and IL-5**

Red rash caused by the T cells that are present and activated (shown by expression of CD45R0 and MHC2 molecules) and express homing receptors for the skin. In the skin the activated T cells secrete chemokines that attract other inflammatory cells (monocytes and eosinophils) into the skin, perivascular inflammation in the skin causes the blood vessels to dilate (appears as bright red rash).

**Explain why SCID babies must NEVER be given live vaccines.**

**Case 8: MHC Class II Deficiency**

**Summary:** MHC2 molecules inolved in presenting Ags to CD4 T cells. Peptide Ag are derived from extracellular pathogens and proteins taken up into tracellular vesicles or from pathogens such as mycobacterium that persist intracellularly inside vesicles. MHC2 molecules expressed consititively on APCs (B cells, macrophages, DCs). Also expressed on thymus epithelial cells (role in intrathymic maturation of CD4 T cells) and their expression can be induced on other cells (principally IFNg). T cells also express them when activated. Heterdimers consisting of alpha and beta chain, both genes found on short arm of chromosome 6. DP, DQ, DR. Highly polymorphic. Gene expression strictly coordinated and under complex regulatory control.

Autosomal recessive. Health problems show up early in infancy. Present with mild form of combined immunodeficiency (have increased susceptibility to pyogenic and opportunistic infections). Differ from SCID infants in that they have T cells which can respond to nonspecific T cell mitogens (PHA) and to allogenic stimuli. Progressive infection with attenuated live vaccine BCG not observed in MHC2 deficienct patients (for TB) because mycobacterial Ag derived from BCG can be presented on MHC1 molecules and infected cells can be destroyed by Tc cells.

Highly prone to viral infections. Deficient in CD4 T cells. Moderate to severe hypogammaglobulinemia (polyclonal expansion of B lymphocytes and maturation to Ig secreting plasma cells requires helper cytokines from CD4 T cells, such as IL4)

IFNg induces expression of MHC2 molecules on APCs from normal people but fail to induce their expression on APCs of paitents with MHC2 deficiency (defect might lie in regulation of expression of MHC2 genes). When B cell lines from 2 different patients were fused, expression of MHC2 is often observed (means one cell must be able to replace whatever the other is lacking, carry different genetic defects). Lack of MHC2 molecules results from defects in the TFs required to regulature their coordinated expression. All 4 of the TFs bind to the 5’ regulatory region of the MHC2 genes. 4 different complementation groups (A-D).

**Case:** healthy at birth, pneumonia in both lungs at 6 months, severe cough and feber, tracheal aspirate showed pneumocystic jirovecii, normal T cell proliferative response but T cells failed to respond to tetanus toxoid in vitro although they responded noramlly in thymidine incorporation assay when stimulated with allogenic B cells, serum Igs were low, WBCs were elevated (82% were neutrophils), low lymphocytes but high B cells, low CD4 T cells, normal CD8 T cells (substantial amount of T cells and normal response to PHA ruled out SCID diagnosis), circulating B cells were transformed with EBV and analyzed with FACS, didn’t express HLA DQ or DR

**Treatment:** hematopoietic stem cell transplantation (number of circulating CD4 T cells can remain low because positive selection of donor derived CD4 thymocytes is compromised due to lack of MHC2 cells on the surface of the patient’s thymic epithelial cells), use cyotoxic busulfan and cyclophosphamide to ablate bone marrow and then administer donor bone marrow by transfusion

**Describe the roles of CD4, MHC class II, TCR, antigenic peptide, and CD3 in eliciting activation of T cells in response to conventional antigens.**

Maturation of CD4 T cells in thymus depends on interaction of thymocytes with MHC class 2 molecules on thymic epithelial cells

**Explain that CD4-positive T cells can enhance macrophage activation to allow destruction of intracellular organisms (Th1-type), as well as enhance the production of antibodies by B cells (Th2-type). These activities appear to be controlled by different subsets of helper T (Th) cells releasing different subsets of cytokines.**

**Explain that HLA-DR, DQ and DP represent MHC class II molecules found on antigen-presenting cells, and an inability to express these molecules leads to MHC class II deficiency.**

**Describe the structure of MHC class II molecules as having 2 chains, each of which is anchored in the cell membrane.**

**Explain that MHC class II molecules are expressed constitutively on antigen presenting cells, and that their expression may be induced on other cell types after exposure to IFN- (as may occur in areas of chronic inflammation) and on activated CD4-positive T cells.**

**Explain that infection with *Pneumocystis* pneumonia suggests a deficiency in T cell-mediated immunity.**

**Explain how bone marrow transplantation may be used to at least partially reconstitute immune function.**

**Explain that lack of MHC class II molecules leads to a greatly reduced number of CD4+ T cells relative to CD8+ T cells, because CD4 cells generally mature via interactions with MHC class II –peptide complexes presented by supporting cells in the thymus.**

**Describe how a deficiency in MHC class II molecules leads to a failure to respond to specific protein antigens, such as tetanus toxoid.**

The T cells, although decreased in number, are normal and not affected by the defect so they can respond to PHA and allogenic stimuli in which the Ag is presented by MHC molecules on the surface of nondefective allogenic cells and doesn’t require processing and presentation by defective cells, the failure of lymphocytes to respond to tetanus toxoid resulted from the fact that there were no cells that could present the Ag on MHC2 molecules to the CD4 cells.

Would reject a skin graft because the T cells could recognize the foreign MHC molecules on the grafted skin cells.

**Case 9: DiGeorge Syndrome**

**Summary:** Thymus is the central lymphoid organ in which T cells develop and mature. Composed of an epithelial stroma that becomes populated with precuros T cells and other hematopoietic cells (such as macrophages and DCs). Thymic stromal cells provide a microenvironement that is essential for attraction, survivial, expansion, and differentiation of T cell precurors. Initially develops in the embryo as an epithelial anlage that gives rise to the thymic stroma. Epithelium derives from the endoderm of the 3rd pharyngeal pouch. Part of the 3 and 4 pouches give rise to the parathyroid glands (closely linked to early thymic development). Mesenchymal cells from the arches is also essential for thymic development (gives rise to CT of thymus and the smooth m. of the heart and major arteries)

TF Tbx1 has central role in development of the pharyngeal apparatus and its derivates (including the thymus, parathryoid glands and develping heart). Belongs to a family of TFs that have common DNA binding sequence (T box). T box factors have role in early embryonic cell fate decisions and regulation of development of many embryonic and extraembryonic structures. Tbx1 is expressed in the endoderm of the 3rd pharyngeal pouch and adjacent mesenchyme. Regulates the expression of several GFs and TFs important for development of thymus and parathryoid glands. Controls segmentation of embryonic pharynx and is also required for growth, proper alignment and septation of the cardiac outflow tract.

Deletion of mutation of the TBX1 gene leads to wide range of congenital defects (including thymus development). Is one of more than 35 genes located at chromosome 22q11.2. Most deletions are spontaneous and arise as result of an aberrant meiotic exchange event. Microdeletion of this region is the most common cytogenic abnormality associated with DiGeorge Syndrome and velocardiofacial syndrome, or conotruncal anomaly face syndrome. 90% of patients with the deletion share the same 1.5 Mb or 3 Mb monoallelic microdeletion. Hemizygous deletion can be diagnosed by FISH. 50% of patients with DG syndrome don’t carry the 22q deletion. A minority of patients with DiGeorge carry point mutations of TBX1 gene without the 22q11.2 deletion. Others have deletion on chrom 10.

DiGeorge Syndrome is characeriszed by congenital heart defects, absence or underdevelopment of thymus, hypoparathyroidism, hypocalcemia due tp absent or low parathryoid function, structural defects of face and pharynx (low set ears, hypertelorism-increased disease between eyes, small mouth, and underdeveloped jaw-micrognathia), developmental delay, palatal dysfunction, feeding difficulties, and a variable degree of immunodeficiency (doesn’t correlate with presence or severity of other clinical features). Neurobehavioral and psychiatric abnormlaities (schizophrenia) observed in significant fraction of patients especially during adolescence or adulthood.

Incomplete DiGeorge Syndrome: small thymus and milder immune defect characterized by mild to moderate decrease in T cell counts, but intact T cell function, In most patients some thymic and parathyroid tissue is pressent and these tissue undergo expansion after birth leading to progressive correction of hypocalcemia and some significant T cell development

Complete: complete absence of functional thymus and profound T cell lymphopenia, SCID with increased susceptibility to opprotunistic infections, tend to die by age of 1-2 yrs. if not treated (thymic transplantation). Less than 1% of all patients with DiGeorge.

Atypical: patients may develop expansion of small number of T cell clones and display severe skin rash and lymphadenopathy leading to a phenotype that looks like Omenn syndrome. Variable (or even increased) numbers of circulting T cells with the CD45R0 activation marker are detected and T lymphocytes infiltrate the skin and other organs. Autoimmunity especially leading to reduction in blood cells (cytopenia) is a sign of immune dysregulation frequently seen in patients even after thymic transplant. Thymus has crucial role in tolerance, especially via expression of TF Aire by medullary thmic epithelial cells which induces the expression of peripheral tissue Ag in the thymus to delete self reactive thymocytes. Severe defects of thmic stroma (like DG syndrome) may lead to a reduced expression of Aire and of peripheral tissue Ag and allow survival of self reactive T cells. Thymus is also the tissue in which natural reg T cells are generated (suppress autoimmune manifestations in the periphery, reduction could contribute to autoimmunity).

CHARGE syndrome is a similar phenotype. Due to mutations in gene for chromodomain helicase DNA binding protein 7. Coloboma (small structural defects) of the eye, heart defects, atresia of the choane (blockage of nasal passages), retardation of growth and development, genital and/or urinary abnormalities, and ear abnormalities. These patients may also present with immunodeficiency. Must be considered in differential.

Mutations of the FOXN1 gene (encodes TF for thymic epithelial cell development) accounts for another rare immunodeficiency with very severe T cell lymphpenia. Present with generalize alopecia and lack of hair follicles (nude mouse phenotype).

**Case:** newborn with low birth weight, dysmorphic facial feature (low set ears, small mouth, undersized lower jaw), feeding difficulties, rapid breathing, increased fatigue, bluish discoloration of skin, truncus arteriosis (single common outflow tract from the heart), seizures, low blood calcium due to low parathormone (made by the parahtyroid glands to regulate calcium and phosphorus), no thymic tissue, FISH showed deletion of 22q11.2, low absolute lymphocyte count, almost no T cells, normal CD19 B cells and CD16/56 NK cells, peripheral blood mononuclear cells responded poorly to PHA mitogens and concanacalin A (indicative of poor T cell function, lead to diagnosis of complete DG syndrome), at age 6 developed purple bruises (purpura) and pinpoint red lesions (petachiae) on skin, low platele count due to destruction of platelete by autoantibodies resulting in insufficient blood clotting and bleeding into the skin (immune thrombocytopenia, treat with IV gamma globulin)

**Treatment:** thymic transplant (doesn’t have to be HLA identical, could be that DCs from the host go to the thymus and mediate positive selection of newly generated thymocytes), prophylatic antibiotics to prevent infection with opportunistic pathogens (pneumocystis jirovecii), Ca supplementation

Wouldn’t use bone marrow transplant because the defect is in the thymic epithelium (not hematopoietic cells). Bone marrow transplant has been successful in patients with DG who have received a transplant from HLA identical siblings (bone marrow isn’t manipulated and the mature T cells in the graft expand in the recipient and proivde immune reconstituition, but no new T cells are generated in the thymus)

**Explain the importance of the thymus in T cell development.**

**Explain how a deleted or mutated copy of TBX1 leads to the phenotype of DiGeorge syndrome.**

**List the diagnostic criteria for DiGeorge syndrome.**

**Describe the variations of DiGeorge syndrome including complete versus incomplete.**

**Explain the treatment choice for DiGeorge syndrome.**

**Case 12: MHC Class I Deficiency**

**Summary:** MHC1 are expressed on surface of all cells except RBCs. Particularlly abundant on T and B cells, macrophages and neutrophils. Heterodimeric glycoproteins composed of alpha chains that are highly polymorphic (encoded on short arm of chrom 6) and a beta 2 microglobulin chain (common to all class 1 molecules, located on long arm of chrom 15). Bind peptides derived from proteins synthesized in the cytoplasm. Form a complex of peptide and MHC which can be recognized by Ag specific CD8 T cells. Viral proteins made in cytoplasm and degraded by proteasome complexes. Peptides transported to the ER by TAP1 and 2 transporters located in the ER membrane (located in the MHC). ER contains MHC1 molecules which enter as separate alpha and beta chains as soon as they have been synthesized. Peptides are loaded onto the chains. TAP BP (encoded on short arm of chromosome 6) facilitates interaction of MHC1 and TAP1 and 2 to promote loading of peptides.

Involved in immune reactions against virus infections. CD8 T cells terminate cells by recognzing viral peptides carried on the surface of infected cells. They release pore forming perforin and cytotoxic granzymes, as well as inflammatory cytokines like TNFa and lymphotoxin. CD8 cells can also express FasL which engages Fas on target cells. Both processes induce apoptosis in the infected target cells.

TAP mutations are recessively inherited. Can fight some viruses (shown by Ab titers) if the virus could stimulate an increased expression of MHC1 molecules to terminate the infection properly. Patients with MHC1 defiencys often suffer from midline granulomatous disease and vasculitis especially on extremities. Profund reduction of CD8 T cells as a direct consequence of lack of MHC1 molecules on surface of epithelial and DCs in thymus. Interaction of thymocytes with MHC1 molecules expressed by thymic epithelial and DC s is essental for intrathymic maturation of CD8 T cells.

Repeated respiratory infections caused damage to the airways resulting in bronchiectases. Abundant Haemophils and pneumocicci is characteristic of patients with bronchiectasis and not due to immune defiency against capsulated bacteria.

**Case:** 17 yr. old girl with severe bronchiectasis (dilation of bronchi from repeated infections) and persistent cough that produced yellow-green sputum, had been chronically ill since age 4 when she started getting repeated sinus infections, middle ears, and lungs due to respiratory viruses, h. influenzae and strep pneumoniae cultured from sputum, brother age 7 also suffered from chronic respiratory infections since an early age, also had severe bronchiectasis and H. influenzae in his sputum, both had emigrated from Russia to get better medical treatment, had 3 other siblings that were healthy, both had tolerated oral poliovirus, diptheria, pertussis, tetnus, and BCG immunizations and tolerated them well, both had elevated IgG levels, normal B cells and NK cells, deficient in CD8 cells, normal neutrophils and complement, cell mediated immunity seemed normal with delayed hypersensitivity skin tests to TB and candida Ag (not surprising because delayed type hypersensitivity reactions are provoked by Ag specific CD4 T cells), high titers of Ab against mumps, chickepox, measles, influenza (low) and EBV (low), no MHC1 molecules found, homozygous for the MHC region, transformed B cells with EBV showed normal mRNA for MHC1, TAP2 nonsense mutations found (parents were heterozygous)

All CD8 T cells were gamma delta because they are independent of MHC1 expression whereas ab CD8 T cells occurs in thymus and is dependent on expression of MHC1 molecules

**Illustrate the structure of MHC class I molecules and explain how multiple MHC I types are expressed on cells.**

**Explain how MHC haplotypes are inherited.**

**Describe how Fas + FasL and MHC class I + TCR need to interact to enable cytotoxic T cell-mediated killing.**

**Explain that cytotoxic T cells release perforin and granzymes to kill target cells (Fig 5.1).**

**Explain the function of TAP1:TAP2 transporter proteins, how they are encoded within the MHC, and how deficiencies in either TAP1 or TAP2 can lead to MHC class I deficiency.**

**Describe the role of the proteasome in providing peptides for display by Class I MHC molecules.**

**Explain that MHC molecules are not expressed on the cell surface unless they are displaying peptides, and that lack of sufficient peptides leads to greatly diminished display of MHC molecules.**

**During the maturation process, explain how CD8 T cells bearing TCR  receptors interact with MHC class I molecules within the thymus, and how these CD8 cells will not be able to mature if MHC class I molecule expression is greatly reduced on the thymic cells. Note that some T cells with TCR receptors may form, but this receptor matures independently of MHC class I molecules.**

**Explain that delayed-type hypersensitivity skin test reactions are CD4 T cell-dependent and rely on presentation of antigenic peptides in MHC class II molecules (e.g., DTH skin tests are normal in patients with MHC class I deficiency).**

**Explain that CD8 T cells suppress B cell responses to antigen, so that patients lacking CD8 T cells would possess abnormally high levels of IgG.**

Factors that help B cells mature and secrete Igs are derived from CD4 cells which were normal. Factors to suppress B cell mediated humoral immunity are secreted by CD8 cells.